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Gynodioecy in *Salvia omerocalyx* Hayata (Lamiaceae)

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Gynodioecy and floral dimorphism in *Salvia omerocalyx* Hayata, an endangered species endemic to central Japan, is here reported for the first time. In three populations of flowering individuals, one population had approximately equal numbers of hermaphrodites (= individuals only with perfect flowers) and male sterile individuals, another population was composed entirely of hermaphrodites, and the third was hermaphroditic except for two individuals with perfect- and male sterile flowers. Examination of herbarium specimens suggested two other localities with male sterile individuals. Male sterile flowers are significantly smaller than hermaphroditic flowers, but there was no difference in seed set between the two floral morphs. Additional field surveys are needed to determine the exact sex expression of the species.

Key words: Floral dimorphism, Gynodioecy, Lamiaceae, *Salvia omerocalyx*, sexual polymorphism

Salvia L., the largest genus in the Lamiaceae, comprising *ca.* 900 species (Alziar, 1988-1993), is distinguished by its two aborted posterior stamens, and the significantly elongated connective separating the thecae of the two expressed stamens. Diverse mechanisms that promote outcrossing are also known in the genus; *i.e.*, dichogamy (*S. japonica* Thunb., Nakai 1950, *S. verbenaca* L., Navarro 1997), heterostyly (*S. brandegeei* Munz., Barrett *et al.* 2000), and gynodioecy (ex. *S. pratensis* L., Treuren *et al.* 1993).

The Japanese representatives of *Salvia* consist of 10 species and several intraspecific taxa (Murata & Yamazaki 1993). Numerous studies, including pollination (Ohashi 2002, Miyake & Sakai 2005), breeding system (Sudarmono 2007), speciation (Sudarmono & Okada 2007), and molecular phylogeny (Takano & Okada 2011), have been conducted on Japanese *Salvia.* Although breeding systems influence the maintenance of population size, sex expression, which is an important factor in fertility, has not been sufficiently investigated, and all species of *Salvia* are considered to be hermaphroditic.

To establish a conservation plan for the endangered species, *Salvia omerocalyx* Hayata (Japanese name: Tajima-tamurasou), I conducted field observations of several populations of this species. In the process, I discovered both hermaphroditic (= perfect) and male sterile individuals. Here, I present the differences in morphology and seed set between hermaphroditic and male sterile individuals of *S. omerocalyx.* This is the first report of sexual polymorphism in Japanese *Salvia.*

Materials and methods

Salvia omerocalyx is an endangered (Threatened II category, national red list of threatened plants in Japan, Ministry of Environment) perennial herb that blooms from May to July (Murata & Yamazaki 1993). It is endemic to a narrow region on the Japan Sea side of eastern Tottori, Hyogo, and Kyoto prefectures.

I observed flowering individuals in three populations in northern Hyogo Prefecture (Takeno, Inanba, Hyonosen, Fig.1, Table 1), counted all flowering individuals and examined their sexual expression (hermaphrodites *vs.* male sterile flowers). To examine morphological variation between hermaphrodites and male sterile flowers, I

TABLE 1. Study sites and sex expression of *Salvia omerocalyx* in Hyogo Prefecture.

Name	Locality in Hyogo Prefecture	Altitude (m)	No. of individuals examined	Sex expression of each individual			Voucher (HYO)
				HP	MS	GM	
Hyonosen	Fukusada, Yabu-shi	700	23	23	0	0	A. Takahashi 4599
Inanba	Inanba, Hidaka-cho, Toyooka-shi	520	48	46	0	2	A. Takahashi 4596
Takeno	Takeno, Takeno-cho, Toyooka-shi	50	22	12	10	0	A. Takano & K. Chikawa 070523-1

HP=bearing hermaphrodite (=perfect) flowers, MS= bearing male sterile flowers, GM= bearing perfect and male sterile flowers

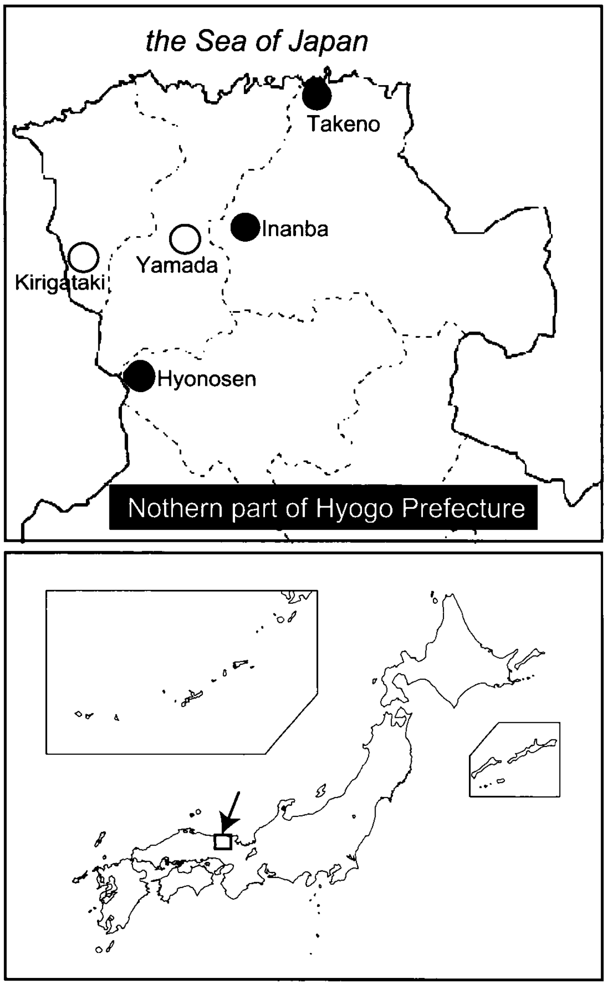

FIG. 1. Maps showing study sites. Upper map shows northern part of Hyogo Prefecture. Closed circles indicate sites of field observations of flowers; open circles show sites where specimens of male sterile individuals were collected. Arrow in lower map indicates the locality of northern part of Hyogo Pref. in Japan.

collected 79 flowers from nine individuals of each sexual type from the Takeno population.

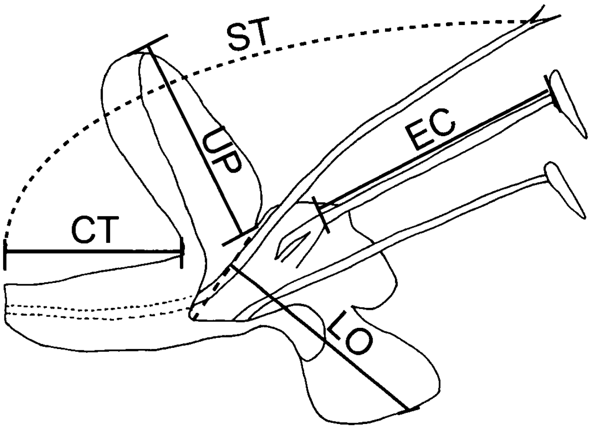

FIG. 2. Measured parts of a *S. omerocalyx* flower. Length of corolla tube (CT), upper lip (UP), lower lip (LO), exerted connective (EC), and style (ST).

The following characteristics were measured with digital calipers: length of the corolla tube (CT), upper lip (UP), lower lip (LO), exerted connective (*i.e.*, the length of the anther connective from the joint in the filament to the fertile thecae, which indicates how far the stamen is exerted from the corolla, EC), and the style (ST) (Fig. 2). I also estimated seed set after anthesis, based on ripening ovules from 9 to 19 calyces collected from 9 hermaphrodite and 9 male sterile individuals. To compare the mean values of hermaphrodites and male sterile flowers, I conducted analyses of variance (ANOVA). Further, I examined herbarium specimens in the Museum of Nature and Human Activities, Hyogo (HYO), The Kyoto University Museum (KYO), and Shoei Junior College (SHO, currently deposited at HYO) to find additional populations where male sterile individuals exist.

Results and Discussion

In hermaphroditic flowers of *S. omerocalyx*, the stamens were extremely exerted from the corolla tube and the thecae contained considerably pollen (Fig. 3. A, C, E). In contrast to hermaphroditic flowers, male sterile flowers showed markedly different stamens: the anther thecae never contained pollen, and the connectives were short-

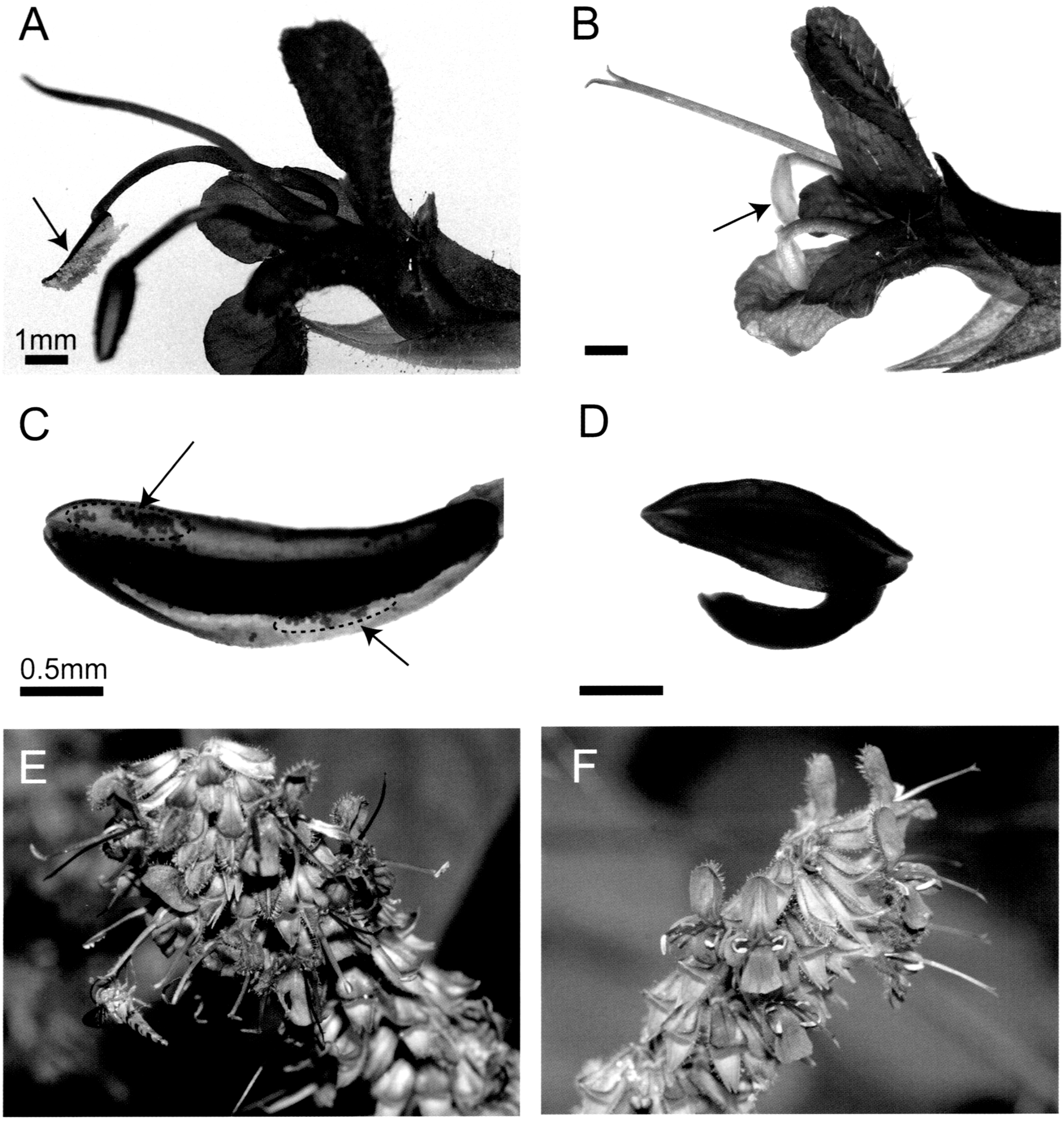

FIG. 3. Two floral morphs of *Salvia omerocalyx*. A, C, E, hermaphrodites, B, D, F, male sterile plants. A–B. Closeup of flower; arrow indicates theca. Pollen is overflowing from theca in hermaphroditic flower (A), but no pollen is visible in male sterile flower (B). Bar = 1 mm. C–D. Closeup of thecae. Pollen is present in hermaphrodite flower (arrows and dotted circle indicate pollen: C), but not in male sterile flower (D). Bar = 0.5 mm. E–F. Inflorescence of hermaphroditic (E) and male sterile (F) plant. *Episyrphus* sp. is eating pollen from exerted stamen of hermaphroditic flower (E). In male sterile plant (F), only style is exerted beyond corolla; no sterile stamens are apparent.

ened (discussed in detail below; Fig. 3. B, D, F).

In total, 22 flowering individuals were found in the Takeno population: 12 hermaphrodites and 10 male sterile ones. At Hyonosen, all 23 flowering individuals were hermaphrodites. At Inanba, of 46 hermaphroditic individuals, two had both perfect and male sterile flowers in their racemes.

Morphological measurements showed that male sterile flowers were significantly smaller than perfect flowers for all parts examined (Table 2). Remarkably, long exerted stamens are a key distinguishing characteristic of *S. omerocalyx* (Fukuoka & Kurosaki 1981) and this study showed that the length of the exerted connective in hermaphrodites was 5.86 (± 0.89) mm. The stamens of male sterile flowers, however, were only 1.59 (± 0.93) mm long and often not exerted beyond the lower lip. At Inanba there were two individuals bearing male sterile and hermaphroditic flowers. The male sterile flowers were smaller than the hermaphroditic ones in the same raceme in all five characteristics measured (data not shown). Seed set was 48.8 (±19.7) % in hermaphrodites and 51.3 (±23.8) % in male sterile individuals; this difference was not statistically significantly (ANOVA, $P = 0.448$).

Among the 156 herbarium sheets at HYO (including those formerly deposited at SHO) and KYO, three were confirmed as male sterile individuals. One was from Takeno (*Takano & Fuse 060527-13*, KYO), and the others were from Yamada Valley at Muraoka-cho, Kami-cho, Hyogo Pref. (*Yanai 12682*, ex SHO, HYO, Fig.1), and Kirigataki W.F., and Shin-onsen-cho, Hyogo Pref. (*Takano et al. 060606-29*, HYO, Fig.1).

This study shows floral dimorphism and sexual polymorphism to be present in *S. omerocalyx*. The ratio of hermaphrodites to male sterile individuals at Takeno (12:10), and the latter two herbarium sheets of male sterile individuals collected from different sites shows *S. omerocalyx* to be gynodioecious. The presence of gynodioecy appears to be particularly common in the Lamiaceae (Darwin 1896). Owens & Ubera-Jiménez (1992) found that 57% of species in the Lamiaceae are gynodioecious. The dominance of hermaphrodites at Hyonosen and Inanba, however, indicates that *S. omerocalyx* might be shifting from hermaphroditism to gynodioecy. Further field studies at more sites during the flowering season are needed to reach a conclusion regarding sex expression in *S. omerocalyx*, but this study confirms that it contains both perfect and male sterile individuals.

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TABLE 2. Floral characteristics (mean ± s.e.) in the hermaphrodite and male sterile flowers of *Salvia omerocalyx* at Takeno population.

	n	CT Length of corolla tube (mm)	UP Length of upper lip (mm)	LO Length of lower lip (mm)	EC Length of exerted connective (mm)	ST Style length (mm)
hermaphrodite flowers	79	6.61 ± 0.74	6.13 ± 0.82	5.60 ± 0.63	5.86 ± 0.89	18.2 ± 1.9
male sterile flowers	79	6.04 ± 0.55	4.72 ± 0.63	4.43 ± 0.65	1.59 ± 0.93	14.8 ± 1.4
ANOVA		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

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